

**What is claimed is:**

1. A method for discrimination of metaplasias from neoplastic lesions in biological samples in the course of cytological testing procedures comprising
  - a. determining the presence or absence of cells overexpressing at least one INK4a gene-product in said biological sample;
  - b. determining the presence or absence of cells expressing at least one further INK4a gene-product in said biological sample;
  - c. assessing simultaneous presence of cells expressing two different INK4a gene-products or the presence of cells overexpressing only one INK4a gene-product alone;
  - d. wherein the simultaneous presence of cells expressing at least two different INK4a gene-products is indicative for neoplastic lesions.
2. A method according to claim 1, wherein at least one of the INK4a gene-products has a molecular weight between 13 and 19 kDa.
3. A method according to claim 1, wherein at least one of the INK4a gene-products is p16<sup>INK4a</sup>.
4. A method according to claim 1, wherein at least one of the INK4a gene-products is p14ARF.
5. A method according to any one of the preceding claims, wherein the INK4a gene-product is a polypeptide or an RNA molecule.
6. The method according to any one of the preceding claims, wherein the neoplastic lesions are lesions of the anogenital tract.
7. The method according to claim 6, wherein the lesion of the anogenital tract is a lesion of the uterine cervix.
8. A method according to any preceding claim, wherein the biological sample is a sample containing cells of anogenital origin.
9. A method according to claim 8, wherein the cells are cells originating from the uterine cervix.

- 10.A method according to claim 9, wherein the biological sample is a cytological or histological preparation of the cervix uteri.
- 11.A method according to any one of the preceding claims, wherein the detection of the INK4a gene-products is performed using at least one probe specifically recognizing the molecules to be detected.
- 12.A method according to claim 11, wherein the probe is detectably labelled.
- 13.A method according to claim 12, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
- 14.A method according to any one of the claims 11 to 13, wherein the probe is a protein and/or a nucleic acid.
- 15.A method according to claim 14, wherein at least one probe is an antibody directed against a INK4a encoded gene-product.
- 16.The method according to claim 15, which comprises an immuno-chemical staining procedure.
- 17.The method according to claim 14, wherein at least one probe is a nucleic acid specifically hybridizing to an INK4a gene-product.
- 18.The method according to claim 17, which comprises an in situ hybridization reaction.
- 19.The method according to claim 17, which comprises a nucleic acid amplification reaction.
- 20.The method according to claim 19, wherein the nucleic acid amplification reaction is PCR or LCR.
- 21.A method according to any of the preceding claims, wherein detection reactions using nucleic acid probes and polypeptide probes are carried out simultaneously.
- 22.A kit for performing the method according to any one of the preceding claims, which is a diagnostic kit or a research kit, comprising at least one or more probes for the detection of the presence or absence and/or the level

of the overexpression of two or more INK4a gene-products in biological samples.

23. A kit according to claim 22, wherein the INK4a gene products are selected from a group comprising p16<sup>INK4a</sup> and p14ARF.

24. The kit according to claims 22 or 23 furthermore comprising at least one of the following

- a. a p16<sup>INK4a</sup> sample for carrying out a positive control reaction
- b. a p14ARF sample for carrying out a positive control reaction
- c. reagents for detection of the presence or absence and/or the level of p16<sup>INK4a</sup>
- d. reagents for detection of the presence or absence and/or the level of p14ARF
- e. one or more samples of INK4a gene-products for carrying out positive control reactions
- f. and one or more reagents for the detection of the presence or absence and/or the level of other INK4a gene products.

25. An immunogenic peptide derived from a cell cycle regulatory protein encoded by an alternative reading frame of the INK4a gene locus, for use in detection and treatment of tumors.

26. The immunogenic peptide according to claim 25, wherein the peptide is selected from a group comprising:

- a. a peptide, which may be predicted as being immunogenic, from the amino acid sequence of the cell cycle regulatory protein;
- b. an HLA-A3 restricted nonamer peptide;
- c. an HLA-A2 restricted nonamer peptide;
- d. an HLA-A\*0201 restricted nonamer peptide;
- e. or a 15-mer peptide

27. An immunogenic peptide according to claim 26, wherein the peptide is selected from a group comprising the sequences given in SEQ IDs No. 1-23.

28. Use of one or more immunogenic peptides according to any one of the claims 25-27 for treatment of tumors.
29. Use according to claim 28, wherein the treatment is selected from a group comprising curative and preventive immunotherapy.
30. Use according to claim 29, wherein the immunotherapy is vaccination therapy.
31. Use according to any one of the claims 28-30, wherein the tumor is selected from a group comprising benign or malignant tumors, carcinomas, sarcomas, leukemias, lymphomas and dysplasias.
32. Use according to claim 31, wherein the tumor is selected from a group comprising cervical cancer, lung cancer, gastric cancer, and colon cancer.
33. Use according to any one of the claims 28-31, wherein furthermore one or more other peptides derived from tumor associated proteins are used.
34. A binding agent directed against the immunogenic peptide according to any one of the claims 25-27, selected from a group comprising
- a monoclonal antibody;
  - a mini-antibody;
  - an antigen binding fragment;
  - an antigen binding peptidomimetic molecule;
  - or a polyclonal antibody
- for use in detection and treatment of tumors.
35. A pharmaceutical composition comprising one or more peptides according to any one of the claims 25-27 and/or one or more binding agents according to claim 34 for the treatment of tumors, wherein the tumor is selected from a group comprising cervical cancer, lung cancer, gastric cancer, and colon cancer and the treatment is selected from a group selected from a group comprising curative and preventive immunotherapy and vaccination therapy.
36. The pharmaceutical composition according to claim 35 comprising furthermore one or more additional peptides derived from proteins, which show non wild-type expression in tumors.

37. The pharmaceutical composition according to claim 36, wherein the additional peptides are derived from proteins selected from a group comprising p16<sup>INK4a</sup>, HPV E6, HPV E7, HPV E2 HPV E4, HPV L1, HPV L2, p27, p21, p15, p19, p53, pRb, MDM2 and peptides encoded by the genes disclosed in the documents WO9904265A2, WO0149716A2, WO0055633A2 and/or WO0142792A2.
38. A method for detection of immunological entities specifically recognizing the peptides according to any one of the claims 25-27 in individuals comprising the steps of
- a. obtaining a sample from the individual;
  - b. contacting the sample with a binding agent binding to said immunological entities selected from a group comprising
    - i. a binding agent directed against said immunological entities
    - ii. a binding agent directed against complexes of the immunological entities together with the respective peptides
    - iii. at least one peptide according to any one of the claims 25-27,wherein said contacting is performed in a way, that binding of the immunological entities to said binding agents gives rise to a detectable signal;
  - c. and assessing the presence or absence and/or the level of immunological entities in said sample from the presence or absence and/or the level of detectable signal.
39. The method according to claim 38, which is used for purposes selected from a group comprising
- a. monitoring in the course of a therapy using peptides according to claim 1-3;
  - b. monitoring in the course of the application of a pharmaceutical composition according to claim 35-37; and
  - c. monitoring in the course of a use according to any one of the claims 28-33.

40. The method according to claim 38, which is used for the diagnosis and monitoring of the disease course of tumors.
41. The method according to any one of the claims 38 - 40, wherein the sample is selected from a group comprising secretions, smears, body fluids, serum, blood, plasma, urine, semen, stool, bile, sputum, biopsies, cell- and tissue-samples, resection samples of tumors, tissue samples prepared by endoscopic means and needle biopsies of organs.
42. A kit for performing the method according to claims 38 to 41 in the course of research studies or in the course of diagnostic procedures.
43. The kit according to claim 42 comprising at least one item selected from a group comprising one or more binding agents according to claim 34, one or more peptides according to claims 25-27 and one or more peptides derived from proteins associated with tumors.